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The Prognostic Value of DNA Content in Patients with Prostatic Carcinoma

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Key Words
Flow cytometry
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Abstract
In 80 patients with pathologically proven prostatic cancer, DNA content was correlated to grade, stage and survival. The survival curve and duration of response to therapy in these patients was examined. At the end of follow-up the cumulative survival curve in the aneuploid patients was 0.40, according to the Kaplan-Meier method, while in the diploid population it was 0.65. Differences between the two groups, aneuploid and diploid, were observed within the various histological subgroups: survival in the G2 population was 0.57 for the diploid and 0.30 for the aneuploid whereas in the G3 patients it was respectively 0.69 for the diploid and 0.05 for the aneuploid patients.

Introduction

Prostate cancer is the most common malignant tumor in men [1]. During the past years the advent of new diagnostic tools has increased the incidence of early diagnosis, but the inability to predict prostatic cancer's clinical outcome, due to the disease's extremely variable natural history [2], remains a dilemma in patient management. In numerous studies the following variables have been used in an attempt to accurately determine tumor aggressiveness: clinical and pathological stage, histological grade, nuclear and nucleolar morphology, tumor volume, steroid hormone receptors, prostate acid phosphatase (PAP) and prostate-specific antigen (PSA) [3–13]. The parameters most often used are tumor grade and stage, this last one however cannot be considered as objective.

Currently, nuclear morphology and cytofluorometry have taken on increased significance due to their objective nature, repeatability and quantitability. Furthermore, molecular biology studies of the genome in prostatic cancer seem to indicate the importance of deletions on the short arm of chromosomes 8 and 10 and on the long arm of chromosome 16 [14, 15].

Our major aim remains that of being able to accurately determine the individual patient's prognosis, in early or later stages of the disease, and its metastatic potential in order to effectively program treatment. The aim of this study was the evaluation of cellular DNA ploidy as an indicator of prognosis in patients with prostatic carcinoma.

Materials and Methods

Patients

One hundred and eleven patients, average age 66.7 years (range 43–86), with pathologically proven prostatic cancer were initially recruited; in 80 of these patients, histological grade according to

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...ofi [16], cellular ploidy and PSA values during treatment ...e available for evaluation. Follow-up periods ranged from 54 ... 102 months. Survival curves were calculated using the ...plan-Meier product limit method [17].

Samples

Transperineal biopsy of the prostate with a Tru-Cut biopsy nee... under sonographic guidance was performed and multiple speci... from both lobes, apex and base were obtained, according to ...dge's technique [18]. These samples were used for histological ...sification according to Mostofi [16] and flow cytometry analysis. The samples for flow cytometric analysis were stored at 4 °C in ...PMI 1640 medium (Gibco, New York, N.Y., USA) supplemented ...h 5% fetal calf serum. First, the samples were washed in physiolog...l solution (0.9% NaCl). Monocellular suspensions were obtained by ...chanical treatment with scissors [19]. The cellular suspensions were ...ed with 70% ethanol and stored at 4 °C. Part of the specimens were ...o used for cytologic examination in an attempt to verify their qual...y for cytometric measurements.

Flow Cytometry Staining

The fixed samples were centrifuged at 200 *g* for 10 min, and the ...llets were resuspended in 5% pepsin solution (pH 1.8) for 10 min ...room temperature, under continuous gentle magnetic stirring. The ...psin was then neutralized with 0.1 *M* Tris buffer solution; the sam...es were centrifuged under the same conditions as before, and the ...llets resuspended in the same buffer. The cells were finally stained ...h a dye solution containing 5 mg/ml ethidium bromide (Serva), ...2.5 mg/ml mitramycin (Pfizer Inc., New York N.Y., USA), and ...5 m*M* $MgCl_2$ in 0.1 *M* Tris buffer (pH 7.4) filtered through a 70-nm ...ylon mesh, and kept at 4 °C for 30–60 min before flow cytometric ...alysis [19].

Flow Cytometry

The samples were analyzed with a PAS II flow cytometer (Partec, ...desheim, Switzerland). The signals were collected and accumulated ...a 512-channel memory. The histograms were stored on a floppy ...sk and analyzed by an original software protocol on a PC-XT IBM ...rsonal computer. Flow cytometric analysis was performed accord...g to the recommendations suggested for DNA cytometry nomen...clature [20]. For each case analyzed, the lymphocytes or a normal ...ostate cell sample of the same patient was used as an external and ...ternal standard of cytometrically diploid DNA content [21]. Sam...es exhibiting one or more subpopulations with abnormal DNA ...ntent were defined as cytometrically aneuploid. The level of DNA ...ntent alteration was expressed in terms of DNA index (DI), calcu...ted as the ratio of the G1/0 modal values between the aneuploid ...d diploid subpopulations. The analysis of DNA histogram data to ...lculate the cell cycle frequencies (%G1/O, %S1, %G2+M) was per...rmed by a cumulative curve graphic method [20] elaborated by ...ftware developed in our laboratory according to the method pro...osed by Baisch et al. [22].

Follow-Up

Transrectal ultrasonography with a 7-MHz probe was performed ...r the evaluation of local extension, whereas for the determination ...lymph node status, computerized tomography and nuclear mag...tic resonance was used, and total body bone scans, abdominal ...trasonography and a plain film of the chest for the determination of ...entual metastases.

RIA, testosterone, LH, FSH, PAP and PSA values were determined before beginning therapy and afterwards for every 3 months. Treatment consisted in the use of LHRH analogue alone or in combination with cyproterone acetate.

Results

Patients were staged according to the AUS classification. Clinically, 6 patients were stage A-B, 4 (66.6%) diploid and 2 (33.4%) aneuploid; 18 patients stage C_1, 12 (66.6%) diploid and 6 (33.4%) aneuploid; 19 patients were stage C_2, 12 (63.2%) diploid and 7 (36.8%) aneuploid. 3 (42.8%) out of 7 D_1 patients were diploid, while 4 (57.2%) were aneuploid; 11 (36.6%) out of 30 D_2 patients showed a diploid pattern, while 19 (63.3%) had an aneuploid pattern.

According to the grading and cellular ploidy, 16 patients (20%) were G1, 14 (87.5%) diploid and 2 (12.5%) aneuploid; 38 G2 (47.5%), 21 (55.3%) diploid and 17 (44.7%) aneuploid; 26 (32.5%) G3, 7 (29.9%) diploid and 19 (73.1%) aneuploid.

The patients with a grade 1 and 2 prostate carcinoma showed the same survival curve, with 52 and 45% of survival after 60 months of follow-up. The survival of G3 patients decreased rapidly from 45 to 5% in the follow-up period and ranged from 40 and 48 months (fig. 1).

If we consider the survival related to the cellular ploidy, the better value is that of diploid patients, with a value of 65% after 60 months; the aneuploid patient survival was 40% after the same follow-up period (fig. 2).

The survival of patients with the same histological grade was studied in relation to cytometric ploidy with the Kaplan-Meier method. In the G1 group the survival was the same for diploid and aneuploid patients, also considering the low number of aneuploid patients in this group. In the G2 group, survival was 57% for the diploid and 30% for the aneuploid patients, with a not statistically significant difference (fig. 3). In the G3 group, survival values were respectively 45% for the diploid and 5% for the aneuploid patients, with a statistically significant difference ($p < 0.05$) between the two groups (fig. 4). Our data confirmed the higher percentage of aneuploid pattern in patients with advanced disease and higher grade (fig. 5, 6). Analysis of cellular DNA content of prostatic carcinomas by flow cytometry has revealed a significantly higher proportion (<0.05) of aneuploidy in advanced stages (C–D) and a similar correlation to loss of tumor differentiation (G grading), while no significant correlation between ploidy and T stage was observed.

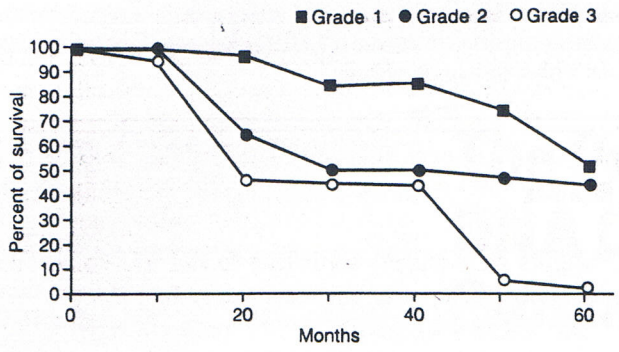

Fig. 1. Cumulative survival curve versus grade in patients with prostatic carcinoma.

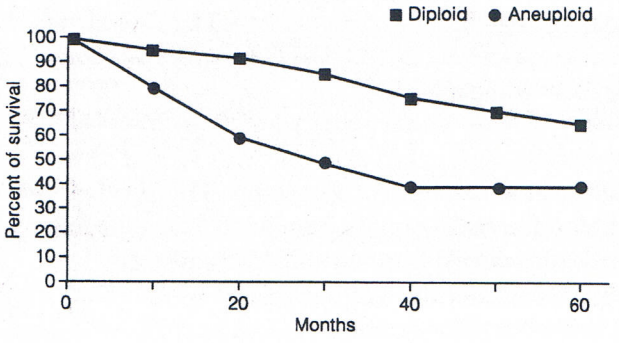

Fig. 2. Cumulative survival curve versus ploidy in patients with prostatic carcinoma.

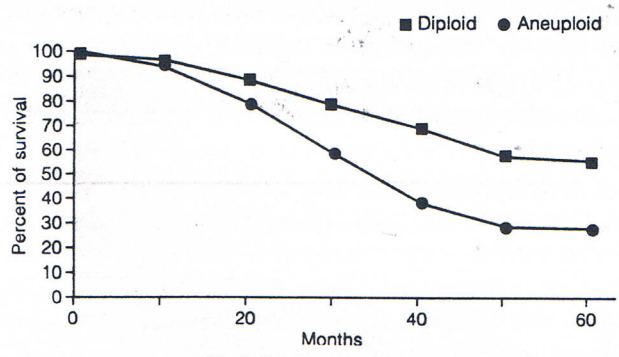

Fig. 3. Survival versus ploidy in G2 patients.

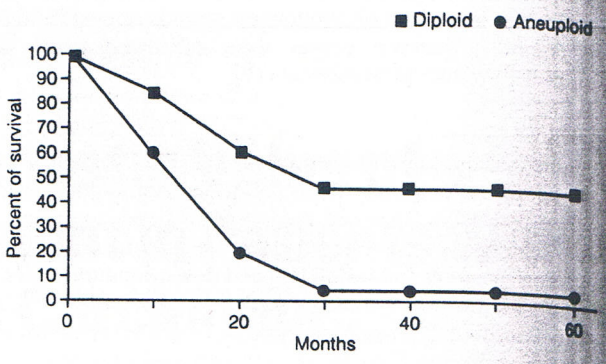

Fig. 4. Survival versus ploidy in G3 patients.

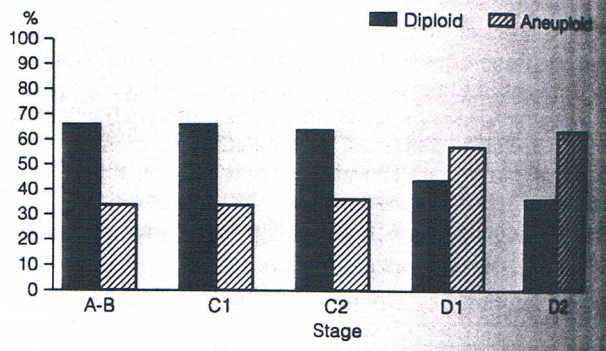

Fig. 5. Ploidy versus staging in patients with prostatic carcinoma.

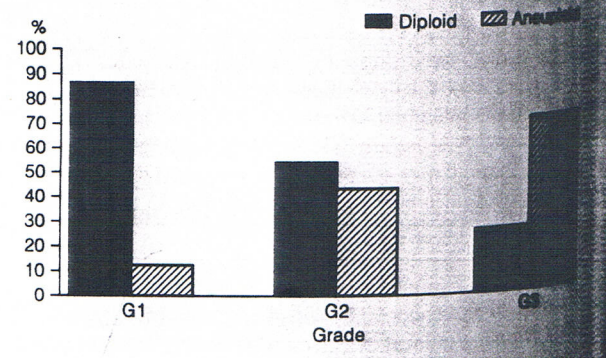

Fig. 6. Ploidy versus grading in patients with prostatic carcinoma.

Conclusions

For the urologist it is always more useful to obtain an early diagnosis because of the increasing incidence of prostatic carcinoma. To reach this goal, with the improvement of technological procedures, results in better choice of proper therapy. A deeper knowledge of the features which may affect the disease's development and their prognostic power values allows for more reliable therapy and decision-making. In the evaluation parameters, cellular DNA content by flow cytometry on the surgical specimens seems to result in a possible discrimination of the cellular clones constituting neoplasm aggression.

The predictive value of this method seems to reach the best reliability in undifferentiated histological tumors (G3), with a statistically significant difference between diploid and aneuploid patients about survival rates. This parameter did not succeed in obtaining an equal reliability in G1 tumor patients. Better results will be obtained by the analysis of a higher number of cases and a correct correlation with other prognostic factors.

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